Insensitivity of Bacteria to Proposed Antimetabolites of d-Alanyl-d-Alanine

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α-Aminoisobutyryl-d-alanine, d-alanyl-α-aminoisobutyric acid, and α-aminoisobutyryl-α-aminoisobutyric acid, proposed as antimetabolites of d-alanyl-d-alanine in bacterial cell wall synthesis, failed to inhibit bacterial growth.

CERTAIN α-methyl derivatives of amino acids function as antimetabolites of the corresponding natural amino acids with consequent inhibition of the enzyme involved in transformation of the natural substrate (1, 2, 4-8, 10). In addition, d-cycloserine, which is considered to be a structural analogue of d-alanine, appears to suppress bacterial cell wall synthesis by inhibition of alanine racemase and d-alanyl-d-alanine ligase (3, 9).

We thus proposed that an α-methyl derivative of d-alanyl-d-alanine may serve as an antimetabolite of the natural substrate with resulting inhibition of the enzyme which inserts this dipeptide into the bacterial cell wall. This would yield osmotically fragile organisms, as are obtained with d-cycloserine, penicillins, and cephalosporins.

α-Aminoisobutyryl-d-alanine (I), d-alanyl-α-aminoisobutyric acid (II), and α-aminoisobutyryl-α-aminoisobutyric acid (III) were custom-synthesized by Cyclo Chemical Co., Los Angeles, Calif. Compound II was anhydrous, whereas I and III were monohydrates. Each migrated as a single spot on thin-layer chromatography in three separate solvent systems. Other analytical data are shown in Table 1.

Each compound was dissolved in water to a final concentration of 2.0% and sterilized by passage through a membrane filter. Sterile 6.35-mm disks of absorbent paper (No. 740-E; Schleicher and Schuell Co.) were impregnated in each solution, and the water was removed under vacuum. The dried disks were placed onto seeded agar plates, using Trypticase soy agar for bacteria, Sabouraud agar for fungi, and Sauton agar for mycobacteria.

No inhibitory action was observed with any of the three compounds when tested against a total of 40 gram-positive and gram-negative bacteria, fungi, and mycobacteria. d-Cycloserine, when tested under the same conditions, yielded zones of inhibition against all bacteria used in the assay. When measured from the periphery of the impregnated disk to the area of uninhhibited growth, these zones ranged from 4 mm with several strains of Pseudomonas aeruginosa to 15 mm with a number of strains of Escherichia coli and Staphylococcus aureus.

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LITERATURE CITED

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